PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 02 August 2000 (02.08.00)	
International application No. PCT/KR99/00699	**Applicant's or agent's file reference** 9FPO-11-03
International filing date (day/month/year) 19 November 1999 (19.11.99)	**Priority date** (day/month/year) 19 November 1998 (19.11.98)
Applicant HONG, Hyo, Jeong et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

15 June 2000 (15.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Olivia RANAIVOJAONA
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

KR9900699

COPY FOR IB

PATENT COOPERATION TREATY

PCT

4

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Artcle 36 and Rule 70)

Applicant's or agent's file reference 9FPO-11-03	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. **PCT/KR99/00699**	International filing date *(day/month/year)* 19 NOVEMBER 1999 (19.11.1999)	Priority date *(day/month/year)* 19 NOVEMBER 1998 (19.11.1998)

International Patent Classification (IPC) or national classification and IPC

IPC7 C07K 16/28, C12N 15/13, C12N 15/63, C12N 15/63

Applicant

KOREA INSTITUTE OF SCIENCE AND TECHNOLOGY et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

☐ This report is also acconpanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demamd	Date of completion of this report
15 JUNE 2000 (15.06.2000)	22 MARCH 2001 (22.03.2001)
Name and mailing address of the IPEA/KR Korean Industrial Property Office Government Complex-Taejon, Dunsan-dong, So-ku, Taejon Metropolitan City 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer HAN, Hyun Sook Telephone No. 82-42-481-5596

Form PCT/IPEA/409 (cover sheet) (July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International aplication No.
PCT/KR99/00699

I. Basis of the report

1. With regard to the elements of the international application:*

☐ the international application as originally filed

☒ the description:
pages 1 -31 , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the claims:
pages _____ , as originally filed
pages _____ , as amended (together with any statment) under Article 19
pages _____ , filed with the demand
pages 32 - 34 , filed with the letter of17 January 2001

☒ the drawings:
pages 1 - 11 , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the sequence listing part of the description:
pages 1 - 12 , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application(under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide** and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained inthe international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disc losure in the international applicationas as filed has been furinshed.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☒ the claims, Nos. 1

☐ the drawings, sheet _____

5. ☐ This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box(Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed." and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION

International aplication No.
PCT/KR99/00699

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	2 - 15	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	2 - 15	YES
	Claims	NONE	NO
Industrial applicability (IA)	Claims	2 - 15	YES
	Claims	NONE	NO

2. Citations and explanations (Rule 70.7)

The document WO 93/16192 A1 is cited in the search report. This publication relates to a humanized antibody capable of binding to a Hepatitis surface antigen, to a pharmaceutical composition which contains the antibody, and to its use in the treatment of Hepatitis and Hepatitis-related disorders. Thus, claims 1 and 15 as originally filed have been anticipated by this state of the art.

However, with the letter of 17 January, 2001 new claims have been filed and the subject matter of the claims 2 to 15 is directed to humanized antibodies specific for HBV surface antigen pre-S1, containing a humanized variable region which comprises specific amino acid sequence described by SEQ ID NOs. 20, 21 or 23. Such humanized antibodies are neither disclosed in the state of art document cited in the search report nor obvious for a person skilled in the art. Therefore, the subject matters of claims 2 to 15, filed with the letter of 17 January, 2001, comply with the requirements of novelty, inventive step and industrial applicability.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07K 16/28, C12N 15/13, 15/63, A61K 39/395 — A1

(11) International Publication Number: **WO 00/31141**

(43) International Publication Date: 2 June 2000 (02.06.00)

(21) International Application Number: PCT/KR99/00699

(22) International Filing Date: 19 November 1999 (19.11.99)

(30) Priority Data:
1998/49663 19 November 1998 (19.11.98) KR

(71) Applicants *(for all designated States except US)*: KOREA INSTITUTE OF SCIENCE AND TECHNOLOGY [KR/KR]; 39-1 Hawolgok-dong, Sungbuk-ku, Seoul 136-130 (KR). KOREA GREEN CROSS CORPORATION [KR/KR]; 227 Gugal-ri, Kiheung-eup, Yongin-si, Kyonggido 449-900 (KR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: HONG, Hyo, Jeong [KR/KR]; #15-401 Kit Apt., 237 Kajeong-dong, Eusong-ku, Taejon-si 305-350 (KR). RYU, Chun, Jeih [KR/KR]; #136-1203 Hanbit Apt., 99 Oeun-dong, Eusong-ku, Taejon-si 305-333 (KR). HUR, Hyangsuk [KR/KR]; #106-1508 Expo Apt., Jeonmin-dong, Eusong-ku, Taejon-si 305-390 (KR).

(74) Agent: LEE, Won-Hee; Suite 805, Sung-ji Heights II, 642-16 Yoksam-dong, Kangnam-ku, Seoul 135-080 (KR).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HUMANIZED ANTIBODY SPECIFIC FOR SURFACE ANTIGEN PRE-S1 OF HBV AND PREPARATION METHOD THEREOF

(57) Abstract

The present invention relates to humanized antibodies specific for HBV surface antigen pre-S1, which show binding affinity similar to mouse monoclonal antibody and which show remarkably reduced immunogenicity since they have less mouse-derived amino acid residues. Thus, the humanized antibodies of the present invention may be useful for the prevention of HBV infection and for the treatment of hepatitis B.

FOR THE PURPOSES OF INFORMATION ONLY

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BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
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CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

What is Claimed is

1. A humanized antibody specific for HBV surface antigen pre-S1, containing humanized heavy and light chain variable regions

2. The humanized antibody of claim 1, wherein the humanized heavy chain variable region comprises amino acid sequence described by SEQ ID NO: 20

3. The humanized antibody of claim 1, wherein the humanized heavy chain variable region comprises amino acid sequence described by SEQ ID NO: 21

4. The humanized antibody of claim 1, wherein the humanized heavy chain variable region comprises an amino acid sequence which is modified from an amino acid residue of SEQ ID NO: 21 by at least one amino acid substitution selected from the group comprising

$Lys^{12} \rightarrow Val^{12}$, $Thr^{28} \rightarrow Ala^{28}$, $Thr^{30} \rightarrow Ser^{30}$,
$Met^{48} \rightarrow Ile^{48}$, $Arg^{67} \rightarrow Lys^{67}$, $Val^{68} \rightarrow Ala^{68}$,
$Met^{70} \rightarrow Leu^{70}$, $Val^{79} \rightarrow Ala^{79}$, and $Tyr^{95} \rightarrow Phe^{95}$

5. The humanized antibody of claim 1, wherein the humanized light chain variable region comprises amino acid sequence described by SEQ ID NO: 23

6. A gene encoding humanized heavy chain which contains a humanized heavy chain variable region of claim 2, 3 or 4

7. The gene of claim 6, wherein the humanized heavy chain variable region comprises amino acid sequence described by SEQ ID NO: 20

8. The gene of claim 6, wherein the humanized heavy chain variable region comprises amino acid sequence described by SEQ ID NO: 21

9. A gene encoding humanized light chain which contains a humanized light chain variable region comprising amino acid sequence described by SEQ ID NO: 23

10. An expression vector containing the gene of claim 6

11. The expression vector of claim 10, pCMV-HKR127HC, wherein the gene of claim 7 is inserted into pRC/CMV (Accession Number: KCTC 0531BP)

12. The expression vector of claim 10, pCMV-HKR127(III)HC, wherein the gene of claim 8 is

inserted into pRC/CMV (Accession Number: KCTC 0691BP)

13. An expression vector containing the gene of claim 9

14. The expression vector of claim 13, pKC-dhfr-HKR127, wherein the gene of claim 9 is inserted into pCMV-dhfr (Accession Number: KCTC 0529BP)

15. Pharmaceutical composition containing the humanized antibody of claim 1, which may be administered in order to prevent HBV infection or to treat chronic hepatitis B

SEQUENCE LISTING

<110> KOREA INSTITUTE OF SCIENCE AND TECHNOLOGY
KOREA GREEN CROSS CORPORATION

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         35                  40                  45

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     50                  55                  60

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 99/00699

A. CLASSIFICATION OF SUBJECT MATTER

IPC[7]: C 07 K 16/28; C 12 N 15/13, 15/63; A 61 K 39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[7]: C 07 K; C 12 N; A 61 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPIL DATABASE, Derwent Publications Ltd., London (GB), PAJ DATABASE, EPO PAJ Database, CA DATABASE, STN Karlsruhe (DE)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 93/16192 A1 (THE WELLCOME FOUNDATION LIMITED) 19 August 1993 (19.08.93), pages 1-5,12-18; claims.	1,15 2-14
A	C.J.RYU et al. "A Humanized Anitbody with Specificity for Hepatitis B Surface Antigen", Hum. Antibod. Hybridomas, Vol. 7, No. 3, 1996, pages 113-122., totality.	1-15
A	C.J.RYU et al. "In Vitro Neutralization of Hepatitis B Virus by Monoclonal Antibodies Against the Viral Surface Antigen", Journal of Medical Virology, Vol. 52, 1997, pages 226-233, totality.	1-15

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
„A" document defining the general state of the art which is not considered to be of particular relevance
„E" earlier application or patent but published on or after the international filing date
„L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
„O" document referring to an oral disclosure, use, exhibition or other means
„P" document published prior to the international filing date but later than the priority date claimed
„T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
„X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
„Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
„&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 2000 (27.01.00)	29 March 2000 (29.03.00)
Name and mailing adress of the ISA/AT Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/200	Authorized officer Weniger Telephone No. 1/53424/341

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR 99/00699

Patent document cited in search report			Publication date	Patent family member(s)			Publication date
WO	A1	9316192	19-08-1993	AU	A1	34595/93	03-09-1993
				GB	A0	9202796	25-03-1992
				ZA	A	9300928	10-08-1994

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BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
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BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
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CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
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CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
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AMENDED CLAIMS
[received by the International Bureau on 19 May 2000 (19.05.00);
original claims 1-15 replaced by new claims 1-21 (5 pages)]

1. (amended)

A vaccine composition for oral administration consisting essentially of protein antigens, in an amount effective to induce an immune response to said antigen, encapsulated in alginate microspheres having a mean diameter from about 0.1 to about 5 μm.

2. The vaccine composition of claim 1, wherein said protein antigen is one or more selected from the group comprising bacterial surface protein antigens, endotoxin or exotoxin antigens, autoantigens, and allergens.

3. The vaccine composition of claim 1, wherein said protein antigen is one or more selected from the group comprising a pnemococcal antigen, a *Hemophilus parainfluenza* antigen, a diphtheria antigen, a pertussis antigen, a tetanus antigen, a enterotoxigenic *E. coli* antigen, a dysentery antigen, a cholera antigen, a gonococcus antigen, a influenza virus antigen, B type hepatitis virus antigen, a measles antigen, a smallpox virus antigen, and a rubella antigen.

4. The vaccine composition of claim 3, wherein said

pneumococcal protein antigen is one or more selected from group comprising pneumolysin, neuraminidase, autolysin, pneumococcal surface adhesion A(PsaA), and pneumococcal surface protein A(PspA).

5. The vaccine composition of claim 1, wherein said vaccine includes additionally immnune adjuvants such as cholera toxin, cholera toxin B subunit, *Escherichia coli* Heat labile enterotoxin, muramyldipeptide or phorbolester.

6. The vaccine composition of claim 5, wherein said immune adjuvant is added to inside or outside of the alginate microsphere.

7. (deleted)

8. The vaccine composition of claim 1, wherein said vaccine causes mucosal immunity response.

9. (amended)

A process for preparing the vaccine composition of claim 1, comprising the steps of:

a) Mixing aliquot of protein antigens or protein antigens including immune adjuvant with a alginate aqueous solution;

b) Homogenizing by adding a mixture of the alginate

and protein antigens of step a) to n-octanol containing an emulsifier;

c) Spraying n-octanol solution containing $CaCl_2$ into the emulsion while stirring the whole emulsion slowly;

d) Adding additional $CaCl_2$ solution to saturate the emulsion, and curing microspheres;

e) Dehydrating microspheres by addition of dehydrating solvent;

f) Collecting the microspheres on membrane filters and washing with alcohol, and then drying *in vacuo*.

10. The process of claim 9, wherein said protein antigen is one or more selected from the group comprising bacterial surface proteins, endotoxin or exotoxin antigens, autoantigens and allergens.

11. The vaccine composition of claim 9, wherein said protein antigen is one or more selected from the group comprising a pnemococcal antigen, a *Hemophilus parainfluenza* antigen, a diphtheria antigen, a pertussis antigen, a tetanus antigen, a enterotoxigenic *E. coli* antigen, a dysentery antigen, a cholera antigen, a gonococcus antigen, a influenza virus antigen, B type hepatitis virus antigen, a measles antigen, a smallpox virus antigen, and a rubella antigen.

12. The vaccine composition of claim 11, wherein said pneumococcal protein antigen is one more selected from group comprising pneumolysin, neuraminidase, autolysin, pneumococcal surface adhesion A(PsaA), and pneumococcal surface protein A(PspA).

13. The process of claim 9, wherein said vaccine includes additionally immnune adjuvants such as cholera toxin, cholera toxin B subunit, *Escherichia coli* Heat labile enterotoxin, muramyldipeptide or phorbolester.

14. The process of claim 13, wherein said immuine adjuvant is added to inside or outside of the microspheres.

15. The process of claim 9, wherein the alginate microspheres solution of step a) is used in 1-5 weight%.

16. The process of claim 9, wherein the emulsifier of step b) is selected from the group comprising HCO-10, HCO-60 and Span-80 which is used in 1-10 weight%.

17. The process of claim 9, wherein the n-octanol containing $CaCl_2$ in step c) is used in 0.5-2 weight%.

18. The process of claim 9, wherein the $CaCl_2$ solution

of step d) is used in 5-10 weight%.

19. The process of claim 9, wherein the dehydrating solvent of step e) is methanol, ethanol, isopropanol or acetone.

20. The process of claim 9, wherein the filter of step f) is a polyvinylidene difluoride(PVDF) membrane filter with a pore size between 0.10-0.44 μm.

21. The process of claim 9, wherein the alginate microsphere is approximately 0.1 to 5 μm in diameter.